Peculiarities of Biological Action of Low Irradiation Doses and Their Probable Relation to the Health State of Participants of Chernobyl Accident Liquidation

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Chernobyl catastrophe is unique not only because of its scale - the amount of released radionuclides, the area of polluted territories, the collective dose obtained by the population and those who took part in the liquidation of accident consequences - but also in connection with extraordinary strong “iodine blow” and existence of millions of people who obtained “low” irradiation doses which had been considered before as absolutely safe. Therefore, to reveal the mechanisms of the action of low dose irradiation and their biomedical consequences is one of fundamental problems solution of which is necessary to develop scientifically grounded criteria of radiation safety.

The latest researches had proved definitively that the low doses cause multiple changes in cells, which remain for a long time after irradiation and provoke changes in their functional activity, and that the processes in cells initiated by low doses differ from ones caused by high doses. At low doses, “dose-effect” dependencies are not linear considerably, indicating incompetence of risk assessment by extrapolation of results from high to low doses.

Therefore, the pre-Chernobyl radiobiological science could not forecast the scales of health disturbance, and could not propose prophylactic measures forestalling the accelerated increase of disease incidence in both adult and children population.

The results of medical observations and fundamental investigations in recent years had changed our attitude considering low doses of irradiation to be absolutely safe and, correspondingly, our attitude towards the problems connected with atomic industry development, radioactive wastes burial and others.

During the activity of the Commission of USSR Supreme Soviet on establishing causes and consequences of the Chernobyl accident, we received reports of different medical institutions on the state of health of participants of the accident liquidation (liquidators) as well as of adult and children population living on radioactively polluted territories. The detailed account of observation materials was presented in the 2nd volume of book “Chernobyl Catastrophe. Causes and Consequences” [1].

In the present paper, we’ll briefly show the principal results. In all reports of 1986-1990, it was told about the changes in health state of liquidators after their work in the radiocontaminated areas. Sseveral hundreds of liquidators were examined in the military surgery clinics of Military Medicine Academy in St. Petersburg. after working at Chernobyl. More frequently found for the first time were hypertonic disease (20.8%), chronic gastritis (14%), neurocirculatory disorders (12.2%), ischemic heart disease (3.7%), chronic hepatitis (1.8%), chronic bronchitis (1.8%), disorder of biliary duct (1.2%) and others. These data are especially verifiable because the Military Medicine Academy had the information on health state of all liquidators (militaly men) for a number of years before the accident. Moscow institutes (MORCI, Institute of Diagnostics and Surgery of Russian Federation Ministry of Health, Institute of Urology of Russian Federation Ministry of Health) also studied the disease incidence of liquidators mobilized by military commissariats. The most frequent were the diseases of endocrine and nervous system, cardiovascular system, digestive organs, musculoskeletal system, and disturbances in male reproduction system. Long-term observation of thyroid state showed changes in its function in 10% of examined people.

Nearly 1,100 liquidators were registered at the Research Institute of Medical Radiology of Armenian Ministry of Health and subjected to dynamic observation. The observation of their health state in the period from 1987 to 1990 showed a tendency of increase of level of nervous system diseases (from 31% in 1987 to 51% in 1990). The increase took place principally for account of organic diseases of nervous system with certain decrease of primarily grown functional disturbances. There were also increases of gastrointestinal and respiratory system diseases. Some regularities of changes were observed in the immune status of liquidators. The examination of patients revealed the immuno-deficit of cellular type, which was characterized principally by decrease of T-dependent link of immunity.

The Ukraine State Register contained 180 thousand of those who participated in liquidation of the accident consequences. The incidence of endocrine and immune systems diseases was characterized by significant annually increase, especially in men. Thus in 1990, as compared with 1988, disease incidence in men grew by 3.7-7.1 times. The dynamics of blood and blood formation organs diseases demonstrated growth of incidence both in men and women: in 1990 it was 5 times as high as it was in 1988. The incidence of diseases of nervous and blood circulation systems increased by 2-3 times as compared with 1988.

We must notice that in that period physicians could not practically reveal the dose dependence for liquidators irradiated in doses 10-20 cGy. In some cases, a group of liquidators who obtained irradiation dose 25 cGy and higher, demonstrated higher incidence of mentioned diseases. However, there were no clear dose dependence on the whole. The largest information in that period was in the All-Union Distributed Register which contained 226.9 thousand of liquidators of Russia, Ukraine and Belarus [2]. According to the Register, the statistically significant growth (1.5-2 times) of general disease incidence of liquidators was observed both in separate countries and in CIS as a whole. For five classes of diseases - 1) nervous system diseases, 2) psychical disorders, 3) blood and blood formation organs diseases, 4) digestive organs diseases, 5) vegetovascular distonia - dose dependence was found: the indices of morbidity of liquidators who obtained dose higher than 30 cGy were significantly higher than those of group with irradiation dose 0-5 cGy. The conclusions about negative effect of irradiation on health state of liquidators were not accepted seriously by medical officials in that period. Many tried to explain things by the increase of diseases «revealing» rather than by the increase of morbidity. Others tried to relate all changes in health state to the results of «radiophobia», emotional stress without any connection with irradiation action. However, in recent years the next conclusions are followed. By the data of National (Russian) Radiation-Epidemiological Register, presently, a growth of morbidity indices has been registered [3] for many classes of diseases both among liquidators and among all irradiated population as a whole. First of all, we must notice continuous growths of indices of malignant neoplasm incidence: in 1990 - 151 per 100,000, in 1991 - 175, in 1992 - 212 (128 - morbidity of liquidators normalized to the age distribution of male population of Russia in given year) and in 1993 - 233 (140). The morbidity indices for malignant neoplasm among liquidators were 50% higher than the corresponding indices of the general statistical data on Russia population in 1992, and 65% - in 1993.

The indices of morbidity of liquidators for endocrine system diseases exceed by 18.4 times the control ones, for psychical disorders - 9.6 times, for blood circulation diseases - 4.3 times, and all classes of diseases - 1.5 times. The morbidity indices dynamics for all these classes of diseases for liquidators has a tendency to increase (Table 1) [4].

The majority of people affected by the Chernobyl accident consequences are suffering changes in the immune system [5]. These changes concern mainly thymus - central organ of immune system - and T-lymphocytes which develop in it. The after-effects of these cells disfunction can be very diverse because they fulfill key functions in protection from viruses and microbes, in anti-tumor resistance, and in immune reactions regulation. Thymus hormones content in blood serum of irradiated people is reduced by 3-5 times in average, which causes suppression of T-lymphocytes protective functions by 3-4 times. The risk of immuno-deficits development increases in the affected people, accompanied by the decrease of immune protection and the increase of frequency of development of malignant tumors and infectious diseases. The immunological changes in the Chernobyl accident sufferers are similar to the manifestation of immune system ageing [5].

Thus, the fact of serious change of health state of liquidators is undoubted. However, disagreement exist

Table 1 Incidence of 12 classes of disease among liquidators per 100 thousand people [4]

Classes of disease	1986	1987	1988	1989	1990	1991	1992	1993
Infectious and parasitical	36	96	197	276	325	360	388	414
Neoplasm	20	76	180	297	393	499	564	621
Malignant neoplasm	13	24	40	62	85	119	159	184
Endocrine system diseases	96	335	764	1340	2020	2850	3740	4300
Diseases of blood and blood-forming organs	15	44	96	140	191	220	226	218
Psychical disorders	621	9487	1580	2550	3380	3930	4540	4930
Diseases of nervous system and sense organs	232	790	1810	2880	4100	5850	8110	9890
Diseases of blood circulation organs	183	537	1150	1910	2450	3090	3770	4250
Respiratory system diseases	645	1770	3730	5630	6390	6950	7010	7110
Diseases of digestion organs	82	487	1270	2350	3210	4200	5290	6100
Urogenital system diseases	34	112	253	424	646	903	1180	1410
Diseases of skin and hypodermic tissue	46	160	365	556	686	747	756	726

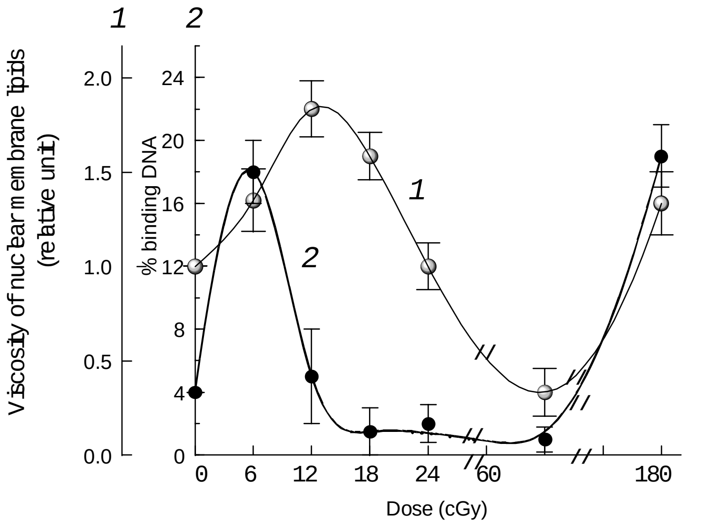

Fig. 1 Percentage of spleen DNA binding with cellulose nitrate (CN) filters (1) and microviscosity of lipids of nuclear membranes of liver (2) of irradiated mice (irradiation rate: 6cGy/day).

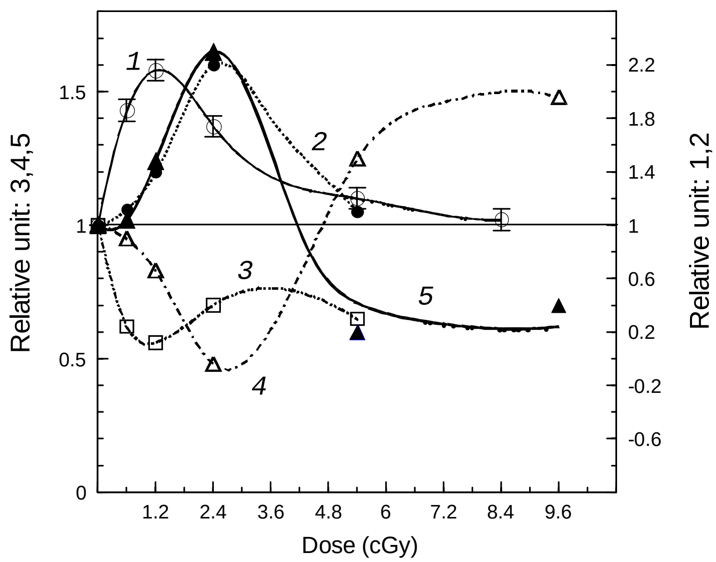

Fig. 2 Dose dependencies of alteration of different structural parameters of genome and nuclear membranes of organs of irradiated mice (irradiation rate: 0.6 cGy/day).

1. DNA retention with CN filters
2. Content of MIF-1 fragment in spleen DNA
3. Alkali elution constant of lymphocytes DNA
4, 5. Rotational correlation time of spin probes

about immediate cause of diseases; the effect of radiation or of psycho-emotional reactions after working in the accident zone. Presently the international organizations (WHO, IAEA) recognize as the main cause of increase of thyroid cancer in liquidators and children population after the accident their irradiation with radioactive iodine, I-131. The rest of diseases, they suppose, are provoked by psycho-emotional reactions.

It is widely known that irradiation not only causes specific reactions but also has «stress» component. Probably, like as specific reactions, «stress» reactions are connected with dosage and dose rate of irradiation. However, while «dose-effect» dependencies are well enough studied for death of irradiated cells, for cell transformation, and for such injuries in DNA molecule as one- and two-thread breaks of DNA-protein joints etc., they are studied quite less for «radiation stress». For a long period, we were occupied in the study of oxidation stress which represents a complex of organism reactions in response to the action of diverse stress factors. Therefore, we undertook investigations of those characteristics as well as of a number of other biochemical and biophysical indices, with regards to their dependencies on irradiation dosage and dose rate. We considered these studies are necessary because changes in active forms of oxygen, lipids peroxidation, antioxidant status of cells, organism organs are important characteristics of pathogenesis, gravity and possibility of treatment, and prognosis of such diseases as malignancy, radiation sickness, neuro-physical disorders, diabetes, cardiovascular diseases, respiratory diseases, digestion and many others [6, 7].

Velocity of alkaline elution of DNA of lymphocytes and liver was studied as well as that of neutral elution and adsorption on cellulose nitrate (CN) filters of spleen DNA, structural (using EPR-method of spin sounds) characteristics of nuclear, mitochondrial, synaptic, erythrocytal and leukocytal membranes. Besides, for the characterization of cell functional activity, the activity and isoforms of aldolase and lactate dehydrogenase enzymes were studied together with the activity of acetylcholinesterase, superoxide dismutase, glutathione peroxidase, velocity of superoxide anion-radicals formation, the composition and antioxidant activity of lipids of above mentioned membranes, and the sensitivity of cells, membranes, DNA, organism to the action of additive injuring factors [8-14].

The bimodal dependence of effects on dose was revealed for all studied parameters. Namely, effects increased at low doses, reached maximum (for low doses), then decreased (in some cases the effect sign reversed) and thereafter increased with the increase of dosage. For example, Figure 1 presents the data on changes of adsorption of spleen DNA and microviscosity of nuclear membranes lipids,

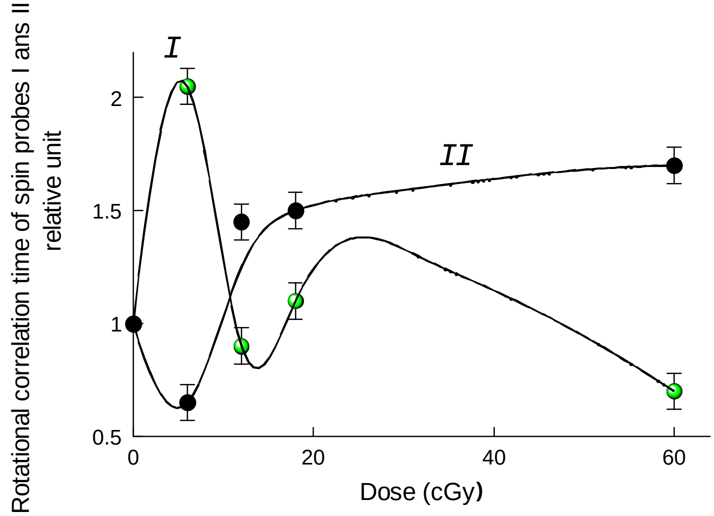

Fig.3 Dose dependencies of alteration of microviscosity of different membrane areas of erythrocytes of mice (irradiation rate: 6 cGy/day).

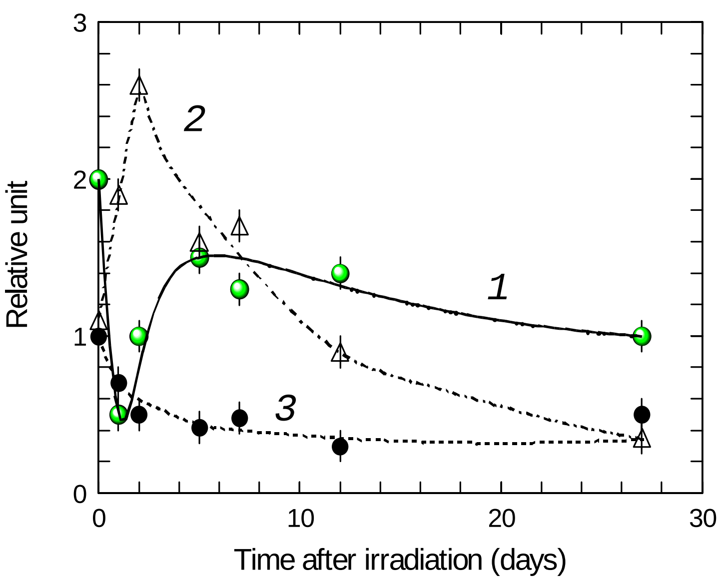

Fig.4 Post-radiation changes of structural characteristics of DNA and nuclear membranes of mice irradiated with 1.2 cGy dose (0.6 cGy/day).

1. DNA retention with CN filters

2,3. Rotational correlation time of spin probes

characterized by the time of rolling correlation of spin sound (I) (ι_1) in dependence of irradiation dose at the intensity 6 cGy/day. Like DNA structural characteristics, microviscosity of membranes lipids changes drastically with irradiation dose, and reached the extreme values were at 6-12 cGy. Note that the level of changes obtained at 6 cGy dose are comparable with changes of structural characteristics of macromolecules at 20-30 times higher doses.

At low-dosage the values of maximum and the dose under which it was reached depended on the nature of studied object and dose rate. In Figure 2 the data are adduced about the changes of genome structural characteristics and nuclear membranes at the irradiation with gamma-rays with lower intensity (0.6 cGy/day). The displacement of maximum to the direction of lower doses with decrease of irradiation intensity is the general regularity of dose dependencies for studied parameters.

The study of genome of irradiated animals by adsorption methods on CN filters in neutral medium permits to observe changes of DNA conformation and to assess the character of recognition of DNA specific sequences by restrictases [15]. As it is seen in Figure 2, at the irradiation intensity of 0.6 cGy/day, the change of adsorption on CN filters of DNA of spleen (curve 1) has the maximum at 1.2 cGy, and at the dose of 5.4 cGy the adsorption value doesn't differ practically from the control one. The results of restriction analysis of DNA of mice spleen using endonuclease EcoRI, expressed in the change of contribution of interspersed repetitions MIF-I in DNA and reflecting the genome structure reconstruction, are represented on curve 2. The dose dependencies of change of mentioned parameters show the extreme character similar to the shift of DNA adsorption maximum to the side of lower doses (1.2 cGy). Analogous but inverse extreme dependence with the minimum at 1.2 cGy was obtained also for the constant of velocity of DNA alkaline elution of mice blood lymphocytes (curve 3).

The changes of nuclear membranes structural characteristics (Figure 2, curves 4, 5) pass over their extremes at the dose 2.4 cGy, while the changes of microviscosity of various areas of membranes are inverse to each other[1]. Unlike the DNA structural characteristics (adsorption), the value of microviscosity of both membrane phases differs considerably from the control in the dose interval of 6-9.6 cGy. The comparable scale of synchronous structural shifts in DNA and membranes under the influence of such low irradiation doses with low intensity deserves attention.

In Figure 3 the data are presented on the change of microviscosity of erythrocytal membranes lipids. Like in all preceding cases, the maximum is observed at low doses. We used the data on changes of kinetic parameters of membrane and cytosol enzymes in cells of irradiated animals for assessing the cells functional activity. The changes of kinetic characteristics of enzymes take place at irradiation doses of 1.2-2.4 cGy.

[1] We used 2 spin sounds; one of them (I) was placed in more hydrophobic area, another - in more hydrophilic one.

Table 2 Changes of ratio of velocity of superoxide radicals generation (VO_2^-) to SOD-activity in liver microsomes and submitochondrial particles(SMP) of irradiated mice (irradiation intensity: 0.6 cGy/day).

Dose, cGy	VO_2^-/Asod $_{microsome}$	VO_2^-/Asod (SMP)
0	1	1
0.6	1.8	1.6
1.2	3.4	0.8
2.4	1.7	1.8
5.4	2.0	1.2

Long-term preservation of altered kinetic properties of enzymes was registered after the irradiation at 1.2 cGy. The disturbance of regulation functions of enzymes was also observed due to changes in correlation between enzyme forms of lactate dehydrogenase and aldolase isoenzyme, and in correlation between enzyme activity and its substrate (superoxide dismutase) (Table 2). Thus, the cells functional activity changes non-linearly with the dose after the low-intensive irradiation. An important factor is the change of sensitivity of separate macromolecules, cells and organism to additive influences of injuring factors both of the same and of other natures. We discovered in our experiments that low-dosage irradiation causes an increase of hemolysis of erythrocytes in mice, and changes the central nervous system sensitivity to the neuromediators, agonists and antagonists. It also changes response of cells to regular influences and repeated irradiation, introducing radiosensitizers and protectors.

The sensitivity of cells of spleen and bone marrow of irradiated animals to the secondary exposure at 6, 7 and 8 Gy doses was studied in the same conditions. It was found that the cells irradiated at low doses have other sensitivity to the secondary exposure [16].

In order to create the scientific bases for forecasting the remote effects of low-intensive irradiation, regularities of early after-effects on macromolecular level were studied. The after-effect of low-dose irradiation to mice, observed in the present study, manifested as alteration of structural characteristics of DNA and membranes (Figure 4) and kinetic parameters of enzymes during all the time of study (27 days) after the irradiation. The post-radiation changes of all macromolecular structures showed non-linear character with gradual return to the control level. Thus, after the irradiation of mice at 1.2 cGy (0.6 cGy/day) which causes the maximum effect, the post-radiation shifts of this parameter were observed.

The data on the change of DNA adsorption and membranes microviscosity after the irradiation at 1.2 cGy dose are compared in Figure 4. The post-radiation changes for lipid sound 1 (curve 2) are expressed stronger than those for sound 2 (curve 3) and inverse to those for DNA as in Figure 2.

The results obtained indicates that there is a close correlation between the processes in mice membranes and genome under the action of low-dosage ionizing radiation both during the irradiation process and afterwards.

We explain the non-linear bimodal dependence of effect on dose on the basis of the idea of the existence of a gap between effects which cause injuries in bioobjects and initialize their recovery systems (Figure 5). In this connection, while the recovery (or adaptation) systems don't function with full efficiency, the effect increases with dose increase, then decreases (or remains on the same level) as the recovery processes become strengthening. Then the effect may be eliminated or may reverse its sign, and increases again with the dose increase when injuries predominate over the recovery. However, in spite of a number of experimental factors confirming such view point, we can't make yet the final conclusion about the mechanism of such dose-effect dependence in the area of low doses of low-intensive irradiation. Alternative explanations of this dependence are presented, e. g. the idea of existence of especial pool of cells sensitive to the action of low-intensive irradiation [17], and a number of others [18, 19].

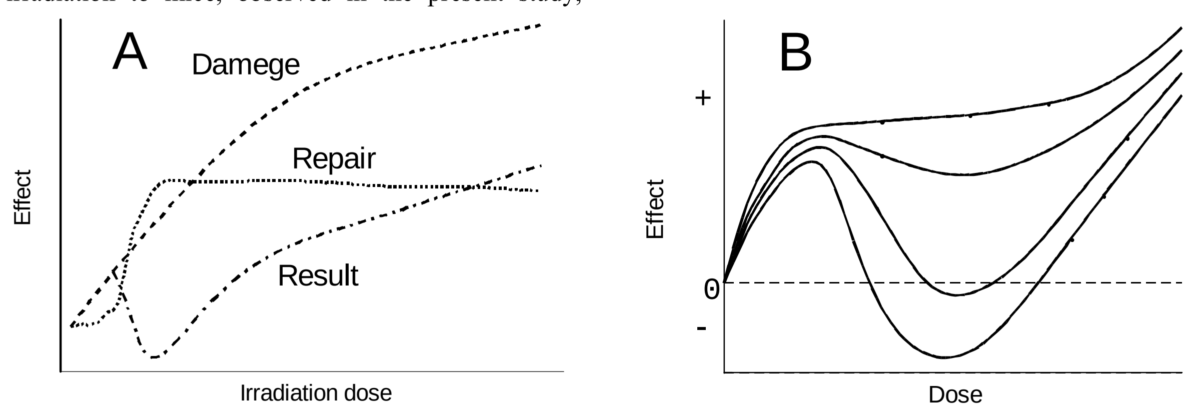

Fig. 5 Scheme of dose dependencies of injury, repaiar and the net effect of irradiation (A) and types of dose-response curves (B).

Table 3 Dependence of different biochemical parameters on irradiation rate.

Criterion	Biochemical parameter	Irradiation dose power, cSv/day	
		0.6	6
Radiation-chemical release for 1 cSv	PB DNA	1	0.3
	ι_{cl} of lipids	$20*10^{-2}$	$7.5*10^{-2}$
	MDA	$9*10^{-2}$	$3*10^{-2}$
Equal-effect doses, cSv	PB DNA	0.6	2.0
		2.4	12
		5.4	30
	ι_{cl} of lipids	0.6	12.0
		5.4	20.0
		9.6	26.0
	MDA	0.6	7.2
		5.4	18.0
		9.6	18.0
Low-intensive maximum (minimum) values	PB DNA	2.2	3.0
	ι_{cl} of lipids	1.2	1.8
	MDA	1.2	2.1
Doses under which the maximum is reached, cSv, and time needed to reach it (days)	PB DNA	1.2 (2)	6 (1)
	ι_{cl} of lipids	2.4 (4)	12 (2)
	MDA	2.4 (4)	24 (4)

Besides, we ought to take into account that, as radiation intensity decreases, the contribution of membranes injuries into the total cells injury increases and there appear new ways of changes in genital apparatus, which are connected with membranes injuries under radiation action.

It is very important to establish the relation between effects at low-intensive dosage and dose rate. But the non-linear and polymodal character of dose dependence makes it difficult to investigate the role of dose rate.

In this connection, we chose several parameters which reflect radiation influences, and studied their changes at dose rate values differing by10 times. Table 3 presents:

1). values of effects per unit dose on initial stages of dose dependencies.
2). doses at which equal effects are observed.
3). values of low-dosage maximum (minimum).
4). doses and time when maximum (minimum) is reached.

Binding percentage of DNA (DNA BP) on cellulose nitrate (CN) filters, properties of viscosity of lipids of erythrocytal membranes (ι_l) and MDA in lipids of erythrocyte membranes were compared. As seen in Table 3, a decrease of irradiation dose rate results in a increase of «radiation-chemical» yield, and in a decrease of the doses under which the equal effects and maximum values of studied characteristics are observed.

The fact of inverse dependence on dose rate in the changes of indices related directly (MDA) or indirectly (ι_l) to lipids peroxidation of membranes is not surprising because theoretical calculation for radiation-chemical yield of peroxides resulted from irradiation gives an inverse dependence of effect on dose rate [20].

All these data show that the organism reaction to low-dosage irradiation is a function of irradiation dose, irradiation dose rate, and time since the beginning of irradiation.

Thus, the investigation showed that:

1). dose-effect dependence shows complicated non-linear character for all studied characteristics at low irradiation doses,
2). low irradiation doses change the sensitivity of biomacromolecules, cells, organs, organisms to the action of other damaging factors,
3). long-term after-effects are observed,
4). inverse dependence is observed in a number of changes,
5). membrane is an important critical target at the action of low-intensive irradiation.

The experimental data indicate that all indices of oxidation stress are changing considerably under the action of low-intensive irradiation. The stress reactions at low irradiation doses manifest the same activity as they do at 20-30 times higher doses. The dose-effect dependence for them is also of non-monotonous, non-linear character. We suppose that these changes can be sufficient to cause various diseases in the organism of liquidators and the people irradiated as a result of the Chernobyl accident.

It is not the psycho-emotional stress, but it is the stress component of low-intensive radiation that can be responsible for the change of health state of liquidators irradiated by low doses.

Biochemical, biophysical, cytological and immunological changes in blood of participants of liquidation of the consequences of the Chernobyl APS accident in 1986-1987.

It is of great interest to clear the question of existence of similar regularities in the population of irradiated people. Regretfully, epidemiological examinations are not always implemented on the level permitting to make certain quantitative conclusions. In this connection, we studied the antioxidant characteristics of plasma and cells of blood of liquidators who had worked at Chernobyl after the accident.

In order to study the effect of ionizing radiation on the system of regulation of peroxidation of lipids (LP) of man, the examination of 104 persons who had worked in 1986-1987 in the zone of the Chernobyl accident (liquidators) and 34 persons who had not registered contact with radioactivity («control» group) was held.

The examination of liquidators was carried out in 1992-1993 during the course of routine observation without connection with concrete diseases. All observed liquidators were considered as absolutely healthy people though many of them talked about various complaints during the examination, of general character as a rule (fatigue, irritation, headache, susceptibility to colds and so on).

Liquidators and the control group did not differ in age. The average age of the examined liquidators was 43, and that of the control group was 45. Well tested and standard biochemical and biophysical methods were used for measuring the indices [21-26].

The object of investigation was cubital vein blood obtained in the morning with empty stomach.

The parameters which characterize the state of LP regulation system (antioxidant status of organism) were determined in this study. The results are adduced on Table 4.

The statistical analysis of results was carried out using a number of statistical methods. Values of Student**,** Wilcoxon**,** Mann-Whitney**,** Kolmogoroff's criteria, criterion of signs; and the multi-factor Mahalanobis's distance between the groups (Hotelling's T-statistics) were evaluated.

The liquidators group significantly differs from the control group in the majority of studied parameters. The content of the most active natural antioxidant - vitamin E (tocopherol) in blood plasma and the quantity of recovered glutathione in liquidators differ from those in «control». Significant differences were found also in the content of antioxidant proteins: glutathione peroxidase and ceruloplasmin, as well as in the non-saturation degree of plasma lipids.

The membranes of erythrocytes of liquidators' blood differed also from the «control» in the quantity of LP secondary products (malonic dialdehyde content), membrane lipids fluidity and degree of lipids non-saturation (Table 4).

Thereby, the people subjected to the ionizing radiation action in various degrees have alterations in the LP regulation system after a long time period (5-6 years after the exposure).

It is well known that the physico-chemical system of regulation of cell metabolism by membranes exists in the organism. Principal components of this system are generation of peroxidation radicals of lipids and antioxidants, the lipids composition, fluidity (microviscosity) of membrane lipid content,

Table 4 Parameters of organism antioxidant status in liquidators and «control».

Index	Control (average)	Liquidators (average)	Significance by Wilcoxon
DLpl (double links in plasma lipids), number of DL/mg of lipids. 10^{18}	0.32	0.33	0.017 *
Dlel (double links in erythrocyte lipids), number of DL/mg of lipids. 10^{18}	0.303	0.30	0.000 *
Vitamin E (conventional units)	20.90	19.05	0.034 *
Vitamin A (conventional units)	2.99	2.85	0.056
Recovered glutathione	19.53	22.19	0.001 *
SOD (superoxide dismutase)	125.41	115.11	0.682
GP (glutathione peroxidase)	7.20	8.87	0.001 *
GR (glutathione reductase)	5.12	4.86	0.760
Hem1 (hemolysis of erythrocytes)	7.23	7.19	0.784
Hem2 (hemolysis of erythrocytes after LP initiation)	7.62	7.81	0.830
MDA1 (malonic dialdehyde in erythrocytes)	1.93	2.22	0.008 *
MDA2 (malonic dialdehyde in erythrocytes after LP initiation)	1.95	2.24	0.003 *
ι_{cI} (time of rotation correlation of spin sound I in erythrocyte membranes)	1.08	1.29	0.000 *
ι_{cII} (time of rotation correlation of spin sound II in erythrocyte membranes)	1.94	1.77	0.022 *
CP (ceruloplasmin)	1.23	1.10	0.046 *
TF (transferrin)	0.78	0.80	0.084
Free radicals with g-factor 2.0	0.69	1.08	0.362
Chromosomal aberrations in lymphocytes	0.81	1.15	0.605

* - indices which differ from control by Wilcoxon's criterion with significance level $p<0.05$

membrane-bound proteins-receptors, enzymes, and channel-forming proteins. All these parameters are linked in normal conditions structurally and functionally. A change of one of them provokes changes of the others (Figure 6). The existence of link with negative inverse relation (the growth of speed of lipids peroxidation causes the enrichment of lipids fractions resistant to the oxidation, and vice versa) allows the system to fulfill the function of adaptation of cell, organ, and organism to the change of environment and to injuring factors, ensuring transition of cell metabolism to another regulation level. The time of relaxation of this regulation system depends on the nature of object (organism, organ, membrane) and changes within the range from 10^2 to 10^4 seconds. In cases either of break of the functional link between the indices, of changes of this link nature, or of long-term action of injuring factor, the system does not return to the normal state. The system of regulation of membranes LP is correlated with other cell regulation systems. It is responsible for cell resistance to damaging factors action and is linked with immune system, ageing processes, cancerogenesis, tumors growth, cardiovascular diseases progress, neuro-psychical disorders, etc. [6].

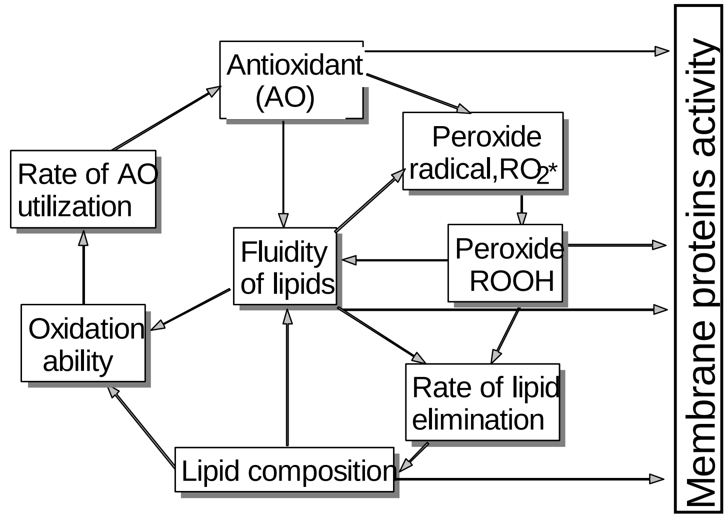

Fig. 6 Scheme of free-radical mechanism in regulation of cell metabolism by membranes.

As it was shown in earlier experimental investigations, during the process of irradiation the concentration of free radicals increases, the number of antioxidants decreases, the lipids enrich with phosphatidylcholine, sphingomyelin, the lipid phase becomes harder (ι_1 increases), and microviscosity of more hydrophilic areas of membrane (ι_2), on the contrary, decreases. The activity of superoxide dismutase, glutathione peroxidase, glutathione reductase changes by stages. Break of connections in the system takes place in a later stage of radiation injury and affects links: AOA-oxidation (changes in contrary directions), ι_1-ι_2 (changes in parallel). Normally, AOA-oxidation changes in parallel, and ι_1-ι_2 do in contrary directions [24].

It was important to determine the changes in the LP regulation system of erythrocyte lipids of liquidators who had obtained doses tens of times lower than those which cause radiation injury. From the experimental data about the existence of close correlation in the change of the regulation system parameters, it was possible to expect an increase of free radical concentration, strengthening of hemolysis, lowering of antioxidants concentration, and increase of hardness (microviscosity) of membranes lipids.

In Table 4, as it was expected, for studied samples of liquidators blood, the concentrations of natural antioxidant - vitamin E and blood antioxidant - ceruloplasmin decreased. The concentrations of free radicals, malonic dialdehyde increased. With the same regularity, the fluidity of different membrane areas changed: ι_1 and ι_2 increased. All this indicates alteration of the antioxidant status of irradiated organism of liquidators.

As far as we are talking about alterations caused by ionizing radiation action, it is important to clear the relation of these alterations with irradiation dosage, Regretfully, we could not get verifiable information on real dose of irradiation of all observed liquidators during their stay in the Chernobyl APS accident zone and, therefore, we could not carry out such analysis on the results of direct dosimetry.

Simultaneously with the study of antioxidant status, the level of chromosomal aberrations in blood lymphocytes was determined in all examined people (by the standard method of study of metaphases of cultivated in vitro lymphocytes). 300 metaphases were studied in the majority of the examined people. The number of chromosomal aberrations, and the number of dicentrics and centric rings were determined among them.

It is known that in a certain range of exposure doses there is a monotonous, practically linearly increasing dependence between exposure dose and the total number of chromosomal aberrations as well as the sum of dicentrics and centric rings number in lymphocytes [24-28], which permits to use the cytogenetic analysis for biodosimetry. We divided thereunder the examined liquidators into several groups in dependence of chromosomal aberration levels and the number of dicentrics and centric rings in their lymphocytes, supposing that thereby we divided them in accordance with their real exposure dose.

As a result, 5 groups of liquidators were formed. The «control» group and the first liquidators group (A group) were composed so that the percentage of chromosomal aberrations in them was not higher than 0.5. The intervals of chromosomal aberrations for another 4 groups constituted respectively 0.5-1 (B group), 1-1.5 (C group), 1.5-2 (D group) and >2 (E group).

Taking into consideration the average level of chromosomal aberrations in lymphocytes of examined liquidators, we may suppose that their average exposure dose constituted 15 cGy (by the number of dicentrics and centric rings - 15 cGy). These calculations confirm the data of Russian Register indicating 15.9 cGy as the average value of liquidators exposure dose in 1986 [3].

These data allow us to consider that the increase of chromosomal aberrations number in groups A-E reflects the increase of dosage obtained by people of these groups.

The data are adduced in Table 5, which characterize the dynamics of alteration of certain indices of antioxidant status in dependence of chromosomal aberrations level in lymphocytes. The change of characteristics in all cases is not of monotonous character. This may be interpreted as an index of complicated dependence of the change of antioxidant status parameters on irradiation dose analogous to one found in experiments.

Table 5 shows that there are differences between these groups in many indices. However, the most interesting is the integral comparison of groups in all set of indices. Such assessment was made using the statistical parameter - Mahalanobis's distance (Hotelling's T-statistics). This method was used many times in the statistical analysis of both experimental and populational multi-factor arrays, and was proved to be successful in the cases when the alteration of a great number of indices in samplings sequence should be reduced to an integral index reflecting all system behavior. In the given case, the control group was compared in succession with all liquidators groups for 15 indices. The most interesting was to compare the liquidators of low (up to 0.5%) level of chromosomal aberrations in lymphocytes with the control group consisting of the people with similarly low level of chromosomal aberrations (up to 0.5%).

It turned out that namely the first group of irradiated people, i.e. those who had the minimal of chromosomal aberrations (<0.5%), by set of indices, differs from the control group significantly (P<0.05) and stronger than other groups do.

If we take into account the mentioned above dependence of number of chromosomal aberrations in lymphocytes on irradiation dosage, we may suppose that the group of liquidators who have insignificant chromosomal aberrations level had obtained low irradiation dose. However, the changes in parameters of their antioxidant status turned out substantial, and obviously indicate the probability of grave and stable disturbance development in the LP regulation system even at low irradiation dose.

The analogous results are obtained in the

Table 5 Mean values of AO indices in the control group and in liquidator groups selected by chromosomal aberrations number.

Indices	Control	Liquidators				
		A	B	C	D	E
DLpl (double links in plasma lipids), number of DL/mg of lipids. 10^{18}	0.34	0.29	0.31	0.31	0.27	0.34
Dlel (double links in erythrocyte lipids), number of DL/mg of lipids. 10^{18}	0.33	0.25	0.26	0.31	0.29	0.30
Vitamin E	23.07	19.80	17.95	20.54	16.13	21.24
Vitamin A	2.99	2.65	2.50	3.20	3.05	3.22
RG (recovered glutathion)	16.70	23.82*	17.57	24.50*	21.98*	25.66*
SOD (superoxide dismutase)	113.12	115.23	120.09	101.08*	136.5	106.76
GP (glutathione peroxidase)	6.91	10.02	9.82	9.26	12.2	7.648
GR (glutathione reductase)	5.61	4.57	5.87	4.66	4.93	4.5
GP-GR (glutahione peroxidase - glutathione reductase)	1.34	2.28	1.91	1.88	2.05	1.97
Hem1 (hemolysis of erythrocytes)	6.78	7.86	11.14*	5.59	7.74	6.70
Hem2 (hemolysis of erythrocytes after LP initiation)	7.27	9.22	10.99*	5.88	6.86	8.17
MDA1 (malonic dialdehyde in erythrocytes)	2.08	2.41	2.74*	1.88	2.67*	1.83
MDA2 (malonic dialdehyde in erythrocytes after LP initiation)	2.07	2.58*	2.58*	2.10	2.88*	1.85
ι_{cI} (time of rotation correlation of spin sound I in erythrocyte membranes)	1.01	1.37*	1.24	1.39*	1.15	1.50*
ι_{cII} (time of rotation correlation of spin sound II in erythrocyte membranes)	2.20	1.51	1.66	1.99	1.48	2.08
CP (ceruloplasmin)	1.16	1.01*	0.92*	1.15	1.18	1.20
TF (transferrin)	0.77	0.82	0.82	0.85	0.72	0.65
Free radicals with g-factor 2.0	0.69	1.20*	1.05	1.02	0.92	1.04
Chromosomal aberrations	0.11	0.18	0.68	1.15	1.66	2.64

*- indices which differ from control by Student's criterion with significance level p<0.05.

correlation analysis of indices of organism antioxidant status in every examined group.

We must note that the observed alterations are not to be considered as some determined pathology. Probably, such alterations in the LP regulation and, consequently, in homeostasis regulation represent so-called pre-nosologic pathology which can remain compensated without manifesting itself clinically, but can realize itself in various diseases in certain conditions. The risk of progress of diverse diseases is to be much higher in the people irradiated even at low doses.

The analogous regularities were found also in the study of immunological characteristics of the same participants of the accident liquidation.

It is interesting to note that we obtained closed dependencies on dose both for immunological index and for antioxidant status. It is very important that the highest deviations are observed in the dose range up to 15 cGy, while these indices are closer to the control ones at the irradiation of 20-25 cGy.

These data confirm once again our idea that the dose dependencies for low-intensive irradiation are of complicated character.

It should be emphasized that the antioxidant and immunological organism statuses are responsible for its resistance to the action of diverse factors affecting health state. The experiments have shown a possibility to control immunological characteristics by changing the antioxidant status in certain direction and vice versa. Therefore, the deficit of antioxidant in the immune systems is a serious index to forecast diseases among persons irradiated at low doses.

Thus, we may suppose that the overall evaluation of such alterations by set of parameters characterizing organism antioxidant status can help to reveal groups of the people with increased risk of different pathologies.

The data obtained in the present study may be considered as one more confirmation of a necessity to apply natural or synthetic antioxidants to the people subjected to the action of ionizing radiation including low doses.

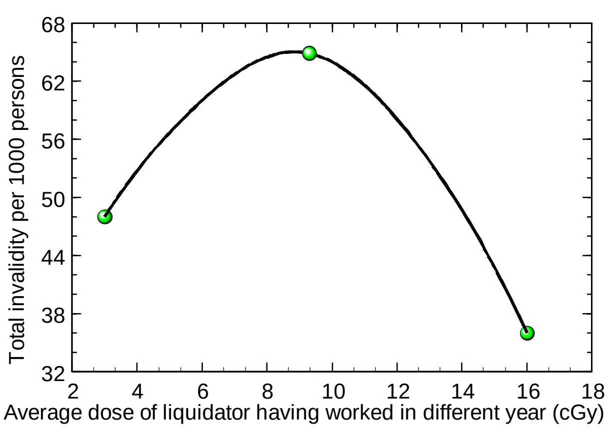

Fig. 7 Dependence of general disability (per 1,000 people) of liquidators on irradiation dose (5 years after their work on liquidation of the ChAPS accident consequences).

Dose dependencies of health state indices of the cohorts exposed to low-intensive irradiation

We tried to compare the alterations of biochemical and biophysical indices of liquidators blood with the regularities of their health state change.

We registered earlier that the increase of incidence of diseases characteristic for the participants of liquidation of accident consequences (liquidators) - vegetovascular distonia, nervous system diseases, psychical disorders, gastrointestinal diseases and others - shows the same tendency (non-monotonous, non-linear dependence on dosage) as obtained in experiments [1]. A number of biochemical and hematological characteristics in the people irradiated at accidents of atomic stations also manifest complicated dependence on dosage [29, 30].

If we consider that the degree of disablement of irradiated contingent is an integral index of health loss, it is important to estimate the rate of disabled people (per 1,000 liquidators) in dependence of obtained dose or the period of work at ChAPS. It is known that liquidators who worked in 1986 obtained the highest irradiation doses (15.9 cGy) in average, in 1987 - 7.9 cGy, and in 1988 and later - 3-4 cGy [3].

Figure 7 shows the data of disabled people among liquidators groups (per 1000) after 5 years since their work at ChAPS. As it is seen in Figure, the dependence is of extreme character with maximum corresponding to 7.9 cGy dose.

However, it would be the most interesting to study such dependencies for remote irradiation after-effects, namely, for malignant neoplasm increase. The question about the effect of low-dosage irradiation on the appearance of malignant tumors and leukoses is widely discussed in the literature. It is known that ionizing radiation can perform both as promoter and as inductor of malignant neoplasm. Increase of dose rate and dosage of irradiation (up to certain limits) causes decrease of promoting and increase of inducing function of irradiation.

Let us consider from these positions the situation with malignant neoplasm by Chernobyl.

Oncological diseases and death rate among the participants of liquidation of the Chernobyl accident consequences are studied in the wide and deep work implemented under the supervision of Academician A. F. Tsyb and published in the Bulletin of National Radiation-Epidemiological Register [31].

Table 6 Morbidity and mortality (per 100,000 man-years) from malignant neoplasms among liquidators [31].

Index	0 - 5 cGy	5 - 10 cGy	10 - 15 cGy	15 - 20 cGy	20 - 25 cGy	25 cGy and higher
Morbidity						
Leukemia	7.68	6.18	8.03	8.48	6.23	3.04
All malignant neoplasm	117.5	122.30	157.51	142.94	134.54	180.56
Malignant neoplasms of digestion organs and peritoneum	21.94	32.26	49.79	38.16	35.60	37.43
Mortality						
All malignant neoplasm	36.20	39.12	44.96	57.95	56.07	40.82
Malignant neoplasms of digestion organs and peritoneum	9.32	15.10	20.87	21.20	24.92	17.0

Table 6 contains the data on the indices of morbidity and mortality from malignant neoplasm among the participants of liquidation of the Chernobyl accident consequences [31]. It is clear that the dose dependence is not monotonous, and for all adduced examples the minimal values of indices of morbidity and mortality correspond to the dose near 25 cGy, while the maximal values are at the dose of 10-15 cGy.

Similar dependencies are not the unique type of dose-effect relation, though they are observed quite frequently. In a number of cases it is regular linear or linear-quadratic dependence. The range of doses under which the decrease of malignant neoplasm or of death rate from them is registered is different for different diseases and depends, beside of the disease nature, on dose rate. For example, the linear dose dependence of mortality from lungs cancer is observed in mortality for lungs cancer of the people irradiated with radon in houses, and of the miners irradiated with radon doses with much more intensity [32, 33].

Many authors consider that the relation between malignant neoplasm and irradiation is worthwhile only in the case when the cancer incidence increases with the irradiation dose. As described above, taking into account all experiments and studies of irradiated populations as well as the data from literature, the PRESENCE of linear or linear-quadratic dependency IS NOT obligatory for the cases of diseases and deaths from malignant neoplasm at low doses and low-intensive irradiation. The absence of monotonous dependence on irradiation dose and the appearance of maxima at lower dose are confirming radiation effects of cancers induction at low doses, rather than refuting it.

As the conclusion of all mentioned, it should be stressed that the regularities of low-intensive irradiation, the low doses effects are the principally new ways of radiation effect on living objects, the new mechanisms of cell metabolism change. The majority of effects are not induced directly by irradiation but indirectly through the regulation system, through alteration of the immune and antioxidant organism status and sensitivity to the action of environmental factors.

It is noteworthy that the similar regularities of the change of studied parameters were observed both in blood of liquidators and in experimental animals. Not only the similar character of dose-dependency but also the identical systemic multifactor response was discovered about the action of low-intensive irradiation.

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